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## Changes in morphotype in the population of *E.coli* in the presence of metal containing nanoparticles

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**Abstract.** The level of variability of the *E.coli* morphotype under the influence of iron oxide nanoparticles (MNPs) depending on their concentration was studied as well as the adaptive capabilities of the microbial population under growth conditions was evaluated. The presence of  $\gamma\text{-Fe}_2\text{O}_3$  nanoparticles in the cultivation fluid affects the cultural and morphological properties of the microbial population of *E.coli* in the process of its development. The appearance of new morphotypes of colonies and cells can be considered as a manifestation of an adaptive mechanism. A change in the morphology of the microbial cell with a high concentration of MNPs leads to abnormal growth and disruption of the division process.

### 1. Introduction

Variability is the main requirement for the formation of adaptation of microorganisms to environmental conditions by the mechanism of the variability of physiological and biochemical functions. Through such mechanism they either activate their vital processes or limit them to a minimum, passing into the stage of anabiosis, which leads to the formation of new races in the structure of microbial populations [1]. It should be noted that the process of variability is multidirectional and this leads to the appearance in the population of phenotypically different clones [2-3].

One of the mechanisms that provide the adaptive capabilities of microorganisms, when exposed to xenobiotics, are changes affecting their morphotype. The viability of microbes depends on the conditions of their habitat as a continuous exchange takes place between them, ensuring the process of microbial metabolism. By cultivating the microbial population in the presence of xenobiotics, their metabolism can be changed. As a result, their cellular organization can be disrupted. The description



of the variability of microorganisms associated with the process of adaptation to environmental conditions in the literature is not complete, especially for such xenobiotics as magnetic nanoparticles.

In this regard, the goal of this work was to assess the level of variability of the *E.coli* morphotype under the influence of metal-containing nanoparticles depending on their concentration and to determine the adaptive capabilities of the microbial population under growth conditions.

## 2. Experimental

Iron-containing nanoparticles (MNPs) are not natural for microorganisms and they do not form a part of the biotic circulation, although iron ions are necessary for their livelihoods. The biological impact may depend on the MNPs composition, average size and surface properties. Iron oxide  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> MNPs were obtained by electrophysical laser target evaporation technique, LTE [4-5] insuring a large size of the MNPs batch [6-7]. The batch size is considered one of the most important parameters in a view of possible biomedical applications [4]. Their structure and magnetic properties were carefully studied by X-ray diffraction analysis, transmission electron microscopy and magnetic characterization. As it was observed in our previous works such MNPs may have core-shell magnetic structure affecting their surface properties and parameters of suspension stabilization.

Experimental studies of the effect of the presence of the iron oxide ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> maghemite) MNPs on the cultural properties of the reference *E.coli* strain included the following steps. The microbial population was grown in a liquid medium (meat-peptone broth, BMP) pH of which was  $7.4 \pm 0.4$ .

Starting from the zero time point, every 24 hours portion of the culture fluid was sown onto solid nutrient medium (meat peptone agar, MPA). Macromorphological characteristics of the grown colonies were recorded, including the size and shape of the colonies. Optical microscopy was used for the shape and size of cells evaluation. The conditions for the cultivation of bacteria in the control and experimental cases were standard. In the control case, the microbial population was grown on a pure nutrient medium. In the experimental case, a suspension of  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> MNPs was introduced into the BMP, in well controlled concentrations of 1.0 and 10.0 maximum tolerated dose (MTD) for Fe<sup>3+</sup>. Maximum tolerated dose was defined as 0.3 mg/l iron ions in water solution. The microbial suspension exposition was 144 hours in all cases under consideration.

Based on the obtained results, periods of microbial culture development were established. For all grown colonies macromorphological characteristics were recorded, including the size and shape of the colonies. Microscopic examination of smears allowed us to recognize the shape, size of cells and their location with respect to each other.

## 3. Results and discussion

According to X-ray diffraction evaluation, the size of the coherent scattering domain dXRD determined by the Scherrer approach based on the XRD patterns was as high as  $21 \pm 3$  nm. The fabricated MNPs were used for fabrication of electrostatically stabilized aqueous suspensions with 5 mM sodium citrate. Following characteristics were obtained for suspension: an average hydrodynamic aggregate size of 86 nm and averaged value of the zeta potential (-42.5) mV. The hysteresis loops of MNPs measured by MPMS XL-7 SQUID-magnetometer for dried suspension samples show no saturation: the magnetization in the field of 65 kOe at room temperature was close to 54 emu/g. The comparative analysis of structural and magnetic parameters confirms consistency of obtained data about close to superparamagnetic behavior for MNPs of this size.

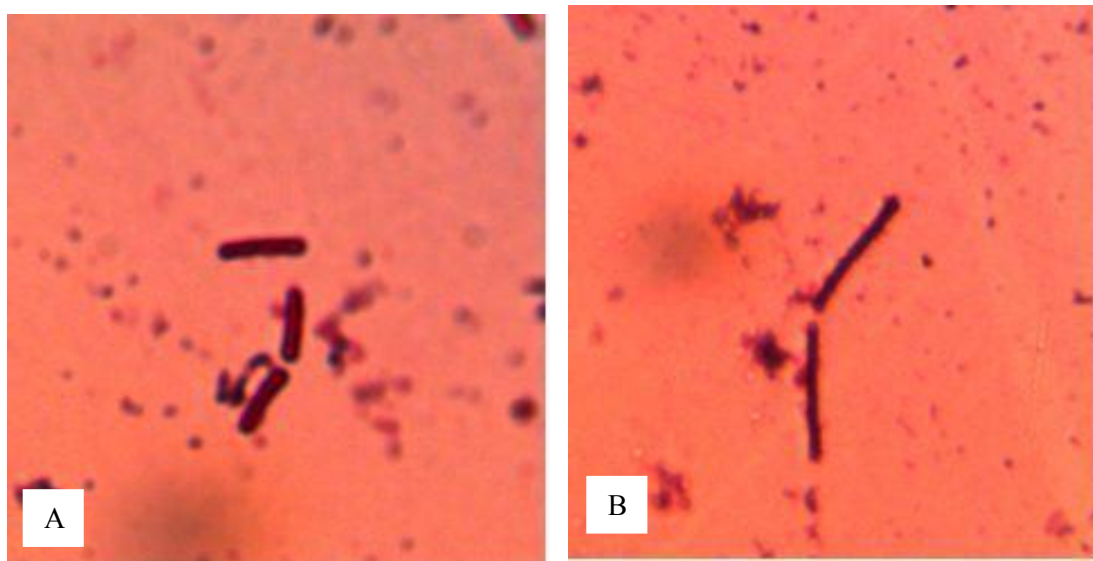
Changes in the size of the microbial culture during periodic growth were identified because of analysis of the morphometric data. The original culture used in the experiment had typical cultural and morphological properties. The cell culture used in the experiment in the initial state had culture-morphological properties typical for the reference *E.coli* strain. In the process of development of the microbial population, it is possible to distinguish the lag-phase period and covers the gap between the inoculation of microbes in the nutrient medium and the beginning of the exponential growth stage. During this period, when sowing a culture on solid nutrient medium, typical colonies are formed in the control sample (figure 1).



**Figure 1.** Round colony of *E.coli* with scalloped edge (control sample, lag-phase) (5 mm x 6 mm).

As a result of microscopic examination of cells forming colonies in the adaptation stage, they found that in the control cells have a rod-like shape typical for *E.coli* with clear boundaries and lie isolated from each other. In the experimental variants in this period of development of the microbial population, the cell morphotype is similar to the control one. It should be noted that on this background, separate large cells appear in the experimental variants, most often at a concentration of 10 MTD (figure 2).

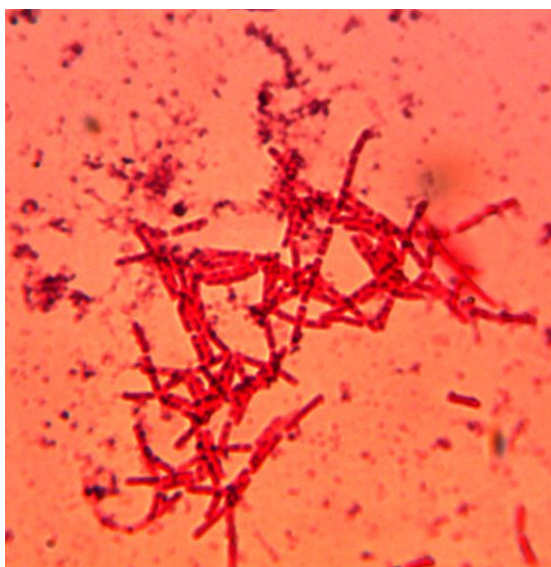
The average size of the colonies in the control was  $4200 \pm 1200 \mu\text{m}$ , whereas in the experimental case with a concentration of 1.0 and 10.0 MTD, the dimensions were  $4000 \pm 1500$  and  $3200 \pm 1800$ , respectively, with a range of 1000 : 8000  $\mu\text{m}$ . It should be noted that, in contrast to the control case, in experimental cases during the lag-phase, the gigantism of bacterial cells was observed, which was especially pronounced when the concentration of iron oxide MNPs was as high as 10.0 MTD. The appearance of *E.coli* cells that were not typical in size indicated an adaptive response of test-cultures for the presence in the environment of  $\gamma\text{-Fe}_2\text{O}_3$  MNPs.



**Figure 2.** Cell morphology (lag-phase): A – control, B - experimental sample with iron-containing nanoparticles of 10 MPD defined for  $\text{Fe}^{3+}$  concentration in water.

In the exponential growth stage, when a microbial culture can be characterized by a maximum speed of division, dependent on the cultivation conditions, bacterial cells are most vulnerable and in some of them, pathological changes form under the experimental conditions can occur. In this phase of the development, colonies appeared in the experiment, differing in dimensional characteristics from the control case. The diameter of the colonies become 1.3 times smaller at a concentration of 1.0 MTD, while at a concentration of 10.0 MTD it increases by 1.3 times.

In the experimental case with an iron ions concentration of 10.0 MTD, large cells are again found, the cut-off area of which is much higher than the typical one. In addition, cells with a smoothed contour are observed, which indicates their non-viability. Cell dysplasia was also noted (figure 3).



**Figure 3.** Experimental case with an iron ions concentration of 10.0 MTD: cell

At the beginning of the stationary growth of the bacterial culture, the colonies in the control case were more uniform in size than during the period of exponential growth. Their average diameter was close to 550  $\mu\text{m}$ . Colonies of bacteria in the experimental case with a concentration of iron of 1.0 MTD were similar to the control case. The average size of colonies in the experiment with a concentration of 10.0 MTD was about 65  $\mu\text{m}$  with a predominance of 12  $\mu\text{m}$  in the structure of the population of small forms.

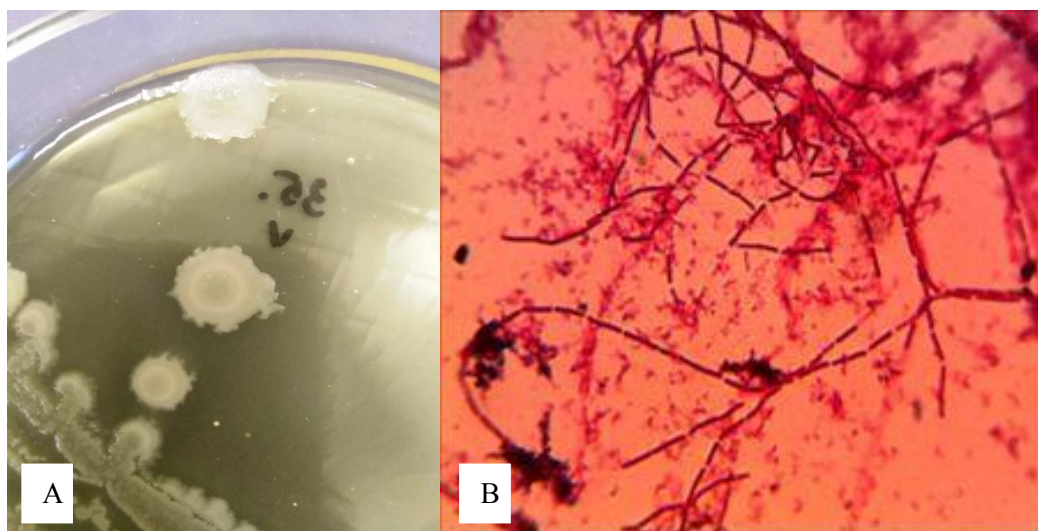
With the accumulation of products of microbial metabolism in the period of stationary growth (approaching 144 hours cultivation time), an increase in the degree of cellular polymorphism was observed both in the control and in the experimental cultures.

The study of the morphology of colonies grown onto solid medium in all analyzed cases did not reveal significant variations. Six types differing from each other in the shape of the colony and the edge morphology were observed. It is noted, the colonies that have an irregular spindle-shaped and filamentary form of colonies can be formed at the stationary stage of growth by typical cells forming small conglomerates that do not stain well and have an internal grain. In a colony with a round shape and scalloped edge, sticks lying in chains were found (figure 4). Colonies with a round shape and a double border consist of elongated sticks, connected in a chain. In addition, there were conglomerates in which the cells acquire a curved shape. The latter form was found in the experiment with nanoparticles at a concentration of 1 MTD (figure 5).

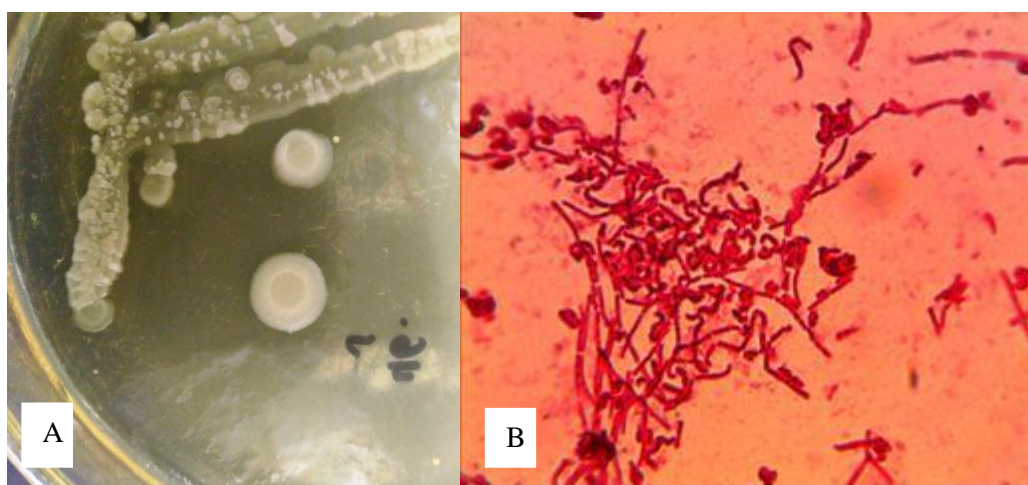
Present study can be viewed as a complementary research to our previous works [2-4, 7-8] in which LTE magnetic nanoparticles were used for testing their interactions with living systems. Obtained phenomenological data are related to very different living systems – from *Exophiala nigrum* strain R-11 unicellular organisms playing an important role in the equilibrium of unique of the Baykal Lake ecosystem, to very important for medical tests post-natal adipose derived multipotent mesenchymal stromal cells. Obtained information provide either useful insight on the features of the



development of microbial population in stress conditions created artificially or reveals degree of the cytotoxicity of used nanomaterials. It is also important to mention the existing ambiguity in determining the MPD parameter in the case of nanoparticles as, strictly speaking, MNPs can not be considered a soluble form of iron.



**Figure 4.** Colonies and *E.coli* cells in the stationary stage of bacterial culture growth: A - a round colony with a scalloped edge (9 mm x 7 mm), B - elongated sticks connected in a chain.



**Figure 5.** Colonies and *E.coli* cells in the stationary stage of bacterial culture growth: A - colonies with a round shape and a double border (9 mm x 9 mm), B - conglomerates of cells and cells of a curved shape, 1 MTD.

At the same time in the present study, we did not have an objective to evaluate the degree of iron accumulation by the living system (neither in ionic form nor as MNPs). There are different reasons for this and quite different methodology should be applied for possible solution of the iron accumulation problem because the difference of micromorphological characteristics can be crucial parameter for such studies.

#### 4 Conclusion

Iron oxide magnetic nanoparticles were fabricated by laser target evaporation technique. The presence in the culture suspension of  $\gamma\text{-Fe}_2\text{O}_3$  MNPs affects the cultural and morphological properties of the microbial population of *E.coli* in the process of its development. The appearance of new morphotypes of colonies and cells can be considered as a manifestation of an adaptive mechanism leading to the formation of quiescent forms, serving as a mechanism for preserving the species. At the same time, the level of change in the cell morphotype in the population of *E.coli* under the influence of  $\gamma\text{-Fe}_2\text{O}_3$  MNPs depends on their concentration. A change in the morphology of the microbial cell with a high concentration of MNPs leads to abnormal growth and disruption of the division process, as evidenced by the formation of involutinal forms among which stand out long filiform, as well as curved or swollen cells.

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